MICROSCOPICAL DIAGNOSIS OF TUBERCULOSIS.

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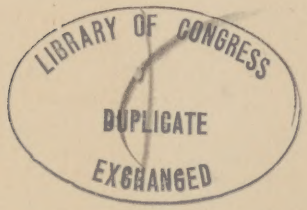

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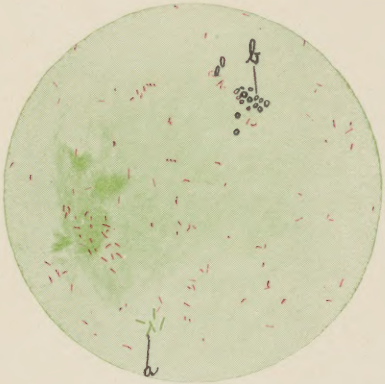


BACILLI OF TUBERCULOSIS

In sputum liquified with a solution of bi-borate of soda; stained with the author's solutions No. 4, *i. e.* A and B of No. 3, modified, and C, as in No. 3. Appearance with objectives ranging from $\frac{1}{6}$ to $\frac{1}{8}$ and with low-power eye-pieces.

a, Foreign Bacilli — *not* of Tuberculosis, stained green with background; *b*, Blackish Bodies, probably Cocci decolorized; they are not uncommon in sputum mounts.

NOTE.—The color of bacilli may vary from pale red to a very dark shade,—almost purple.

PLATE I.

MICROSCOPICAL DIAGNOSIS OF TUBERCULOSIS.

I.—INTRODUCTION.

DURING the past six or seven years, the writer has been engaged very frequently in analyzing material of various kinds for bacillus tuberculosis. During his service at the University of Missouri, and since, in his new field of labor at the Laboratory of Hygiene of Battle Creek,[1] he has had numerous opportunities to test the many methods of microscopical diagnosis of tuberculosis, and to try new ones. His experience has also served in bringing forcibly to his mind the fact that most physicians and medical students suffer unnecessarily the loss of many of the unmistakable advantages which a microscope affords for diagnosis. They labor under the misapprehension that only experts can use this instrument with reliability, and with safety to the patients and practitioners. As a matter of fact, many cases of severe disease could be early diagnosed, and many lives saved, by any physician of earnestness,

[1] Battle Creek Sanitarium.

who would use judgment, and would labor as earnestly for a few minutes with a microscope as he does in searching his text-books, or in attempts at physical diagnosis.

And in no disease is this more true than in tuberculosis. Every doctor can learn to use the microscope with as positive results as this instrument can afford, and every practitioner owes it to his patients to take no chances with their lives; to base a diagnosis only upon evidence, not upon opinions or guess-work. Indeed, in these days of progress and discoveries, a doctor who still guesses at the diagnosis or prognosis of a suspected consumptive, is doing the latter a heartless wrong, and is, in my judgment, guilty of malpractice. And yet we see this every day.

Whatever relation one may think the bacillus of tuberculosis has to the fearful affliction of this name (consumption), one thing is certain: the bacillus is never found in any organ except in connection with this malady. Therefore, when it is present, it always indicates tuberculosis — danger!

In Plates I. and II., here presented, will be seen the appearance of the bacilli of tuberculosis when stained by the writer's method, which will be described farther on in these pages. Plate III. is very highly magnified, and is a rare appearance.

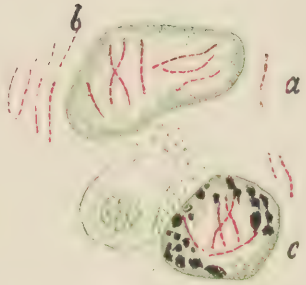
BACILLI OF TUBERCULOSIS IN SPUTUM A FEW DAYS OLD

As they appear under a 1-15 or 1-18 objective.

The specimen had not been treated by any means to destroy the pus cells and mucus. The microbes are stained with the author's solutions A and B, and the field (including pus cells and mucus) with C.

a, Rod-Shape Bacillus; *b*, Pus Corpuscle; *c*, probably a Spore; *d*, a Bacillus, showing dots which are probably Spores; *e*, Strings and Film of Mucus.

PLATE II.



LONGER BACILLI OF TUBERCULOSIS IN SPUTUM.

(Largely magnified.)

Borrowed from Cornil & Babes, "*Les Bacteries.*"

a, Isolated Bacillus; *b*, Bacilli in Epithelial Cell; *c*, Bacilli in a Pigmented Cell.

(Uncommon appearances.)

PLATE III.

II.—THE MICROSCOPE AND OTHER INSTRUMENTS.

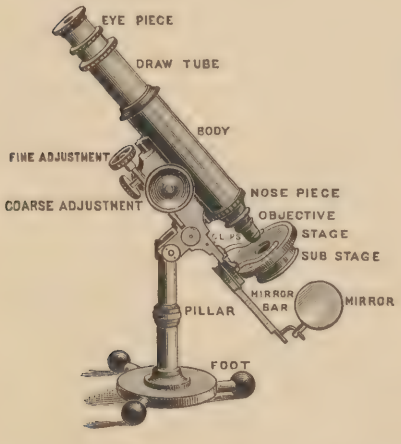
FEW instruments, besides the microscope, are essential for the microscopical diagnosis of tuberculosis. Indeed, one may do the work with it and the few essential cover-glasses and glass slips. But a pair of small forceps and a needle — say a cambric needle fixed in a pen handle — are very useful, and scarcely to be dispensed with; and something to rinse with, such as a wash-bottle or medicine dropper, is necessary. One also needs stains and reagents. It is not necessary to possess a costly microscope, but it is desirable to have a good solid stand, and a clear objective.

The author could not do as well in describing the parts of a microscope as Dr. Whelpley in the following, quoted from *Popular Science News*.[1] As this was written for pharmacists, only the portions essential to our object are quoted, and some other word is substituted for the word "pharmacist:"—

"In selecting an instrument, the first thing for consideration is the stand. With a good stand for work, one is ready to add and make use of such quality

[1] Dr. H. M. Whelpley, F. R. M. S., a well-known scientist and microscopist of St. Louis, Mo.

of optical parts as his requirements may suggest and his means justify; but with a poor stand, the possessor is always at a disadvantage, even with the best of optical parts.



"The *stand* may be defined as a compound microscope without optical parts. Since the optical parts are the only essential portion of a microscope, and we can do no work whatever without them, this may seem like defining a gun as the portion of that firearm without lock, stock, or barrel; but such is not the case. The stand is devised solely for the purpose of using the optical parts to the best advantage. Therefore any one can readily see that the more perfect the stand, the greater the amount of work to be accomplished with the complete instrument, and the more perfect and satisfactory it will be. In considering the various parts of the stand, the accompanying illustration will serve to make them clear.

"The *base*, or lower portion of the stand, first attracts our attention. Instruments can be found with bases of almost all conceivable forms. . . .

"The *feet* forming the tripod may be disguised in the form of the base; but that does not matter, so long as there are three, and only three, points of support. This not only gives the greatest stability to the instrument, with the least tendency to vibrate when the table is jarred, but also has other advantages. . . .

"The *pillar*, or *support*, requires no special attention. With some instruments it is single, while others have a double support, so that the mirror bar swings between them. . . .

"The *joint* seen at the upper end of the pillar is a feature of more importance, and every one should see that his instrument can be inclined to any desired angle.[1]

"The *arm* is not very prominent in our illustration. It is the portion above the joint, and bears the body. In some instruments it is prominent, and closely resembles in form the flexed human arm. If it supports the body firmly, that is all that is required.

"The *body* is supported by the arm, and has attached directly to it the optical parts of the compound microscope. The body varies in size and length

[1] A stiff microscope may do good work, but it is not so convenient. — P.

in different instruments. The size is not of very much importance, but in length it should be what is known as 'standard,' or be so arranged that it can be lengthened out.[1] . . .

"The *draw-tube* is found only in the better class of instruments, and is a provision for adjusting the length of the body. With very high-power objectives it is very essential to have a draw-tube. It should be marked to indicate when the body is 'standard' length. A great convenience is a 'society screw' in the lower end of the draw-tube.[2]

"The *collar* is the ornamental ring, or projection, at the upper end of the draw-tube, or of the body when there is no draw-tube.

"The *nose-piece* is the portion at the lower end of the body. It is provided with a female screw, into which the objective is fastened. By all means purchase a microscope with what is known as the 'society screw,' so that any ordinary objectives can be fitted to it. I have found that the English thread in the 'society screw' is not quite the same as the American, and I was obliged to get an adapter for the use of English objectives on an American stand.

[1] This is not an absolute necessity for our purpose, but is very useful.— P.

[2] To admit various objectives of different makes.

"The *stage* is of importance. . . . It is an item to have a thin stage which admits of oblique illumination in the examination. The expensive mechanical stages are very convenient, but not essential.

"The *sub-stage* must be so arranged that it will admit of the use of sub-stage condenser, polariscope, etc., if one expects to do much *fine*[1] work.

"The *diaphragm* is a contrivance for regulating the volume of light which is admitted to the object. When a sub-stage is present, the diaphragm is adjusted to it; otherwise it is attached to the stage in place of the sub-stage.

"The *mirror bar* and its arrangement is plainly shown in the illustration. . . .

"The *mirror*, if single, must be a concave one. Where there are two, one is plain and the other concave. As far as the plain mirror is concerned, the size does not make much difference, but the larger the concave mirror the better.[2]

"The *clips* are for holding the slide in position.

"The *coarse adjustment* is found on all instruments. The rack and pinion arrangement is the best, and is the one used on the better class of instruments.

[1] The italics are the writer's. — P.

[2] However, some microscopes with very small mirrors do very fine work.— P.

"The *fine adjustment*, or *micrometer screw*, is also a feature of the better instruments, and should be present on every one owned by any one who intends to do much work. It is much more convenient to have the fine adjustment near the coarse one."

To this description of Prof. Whelpley's should be added the optical parts, that is, the eye-piece, or *ocular*, and the *objective*. The latter is the most important part of the microscope. It constitutes the lens or series of lenses attached to the nose-piece, next to the object to be examined, by which the microscopic image is formed. The ocular is the lens or series of lenses next to the eye, which magnify the image formed by the objective, as a hand lens magnifies an image visible to the naked eye.

The cheapest of the outfits generally used in our laboratory for the diagnosis of tuberculosis, represents about the following : —

Microscope stand, objectives, and eye-pieces	$45 00
Pincers	50
Test-tubes and watch-glasses	60
	$46 10

Stains and reagents, such as *aniline oil*, fuchsin, aniline blue or green, alcohol, nitric acid, acetic acid, carbolic acid, liquor ammonia, etc., etc., are

needed according to the methods of staining adopted. These cost a few dollars. But one may purchase stains and reagents already prepared, and avoid the greater cost, and the trouble, and the failures attendant in case of attempts to prepare one's chemicals without sufficient experience and without proper utensils, such as accurate scales, liquid measures, etc. For two or three dollars, one may purchase enough prepared mounting material, including glass slips, cover-glasses, and Canada balsam, to last a year in ordinary practice, while five to ten dollars would scarcely cover the cost of a first attempt to prepare everything one's self. Furthermore, well-prepared mounting fluids, etc., from reliable houses, are usually made from chemically pure drugs, under the guidance of experts.

A dollar Bunsen burner and two feet of rubber tubing may be added to this cost by the physician who may desire to use gas. An alcohol lamp, or even an oil lamp, will answer the purpose. Not much need be spent for a washing apparatus. If neither a medicine dropper nor the stream from a faucet or a dropper be desired, it is easy to improvise a wash-bottle. Every one remembers preparing these as a student, by fitting to a wide-mouthed bottle a cork with two holes holding a glass tube each,— one going to the bottom, and the external extremity bending outside at a sharp angle from the mouth of the bottle in

a downward course; the other penetrating the cork only to the inside of the bottle, and the outward end projecting upward at a very slight angle. The former tube is to throw a jet of water out of the bottle when the mouth blows into the latter. This is used to wash the stain off the cover-glasses in mounting. A medicine dropper is slower, but works fairly well. Water may be used directly from a faucet allowed to run smoothly and thinly, but it is apt to break the glass if the force is great.

A far better outfit, including a Lietz microscope, for instance, with a No. 7 eye-piece corresponding to our $\frac{1}{8}$, or say including one of our popular American or English makes, can be purchased now-a-days for little more than the cost of the above — between $50 and $60. But $30 to $35 will purchase a microscope that will diagnose tuberculosis positively.

NOTE. — Before purchasing a microscope and other equipments, write to optical and surgical instrument houses for catalogues, and examine them closely. I think all of them give a discount for cash, varying from 15 to 25 per cent. See the advertisements in the *Bacteriological World and Modern Medicine.*

III.—PRINCIPLE, OBJECT, AND EFFECT OF STAINING.

THE knowledge of the presence of the bacillus of tuberculosis in an organ, is a positive indication of the dangerous nature of the case presenting it; consequently, any analysis that may reveal it is to be sought, and *should by all means* be sought by every conscientious practitioner. The microscope is the instrument necessary for this analysis.

In examining suspected matter in search of this bacillus, one may find numerous and different germs, particularly if the substance comes from the lungs or throat, as sputum, or from an open wound, as in tubercular joints, or from the fœces, as in intestinal tuberculosis. It is not always possible, then, by a mere glance, no matter how searching, to tell which is the bacillus of consumption and which is not. So investigators set to work to find *differentiating* methods of examination. As a result, numerous processes of staining this particular germ were promulgated. Some of them are very slow; others more rapid; others, again, occupying only a very few minutes. In Chapter VI. will be found the most reliable and the safest formulas in vogue. These stains,

if applied as directed, will be found to stain the bacillus of tuberculosis only or mainly, and to stain the other germs as the background, or leave them as glistening, or yellowish, or brownish, or blackish bodies and debris.

Some staining processes are termed "double-staining," for the reason that they stain the germs one color and the field another; such a one was used in the mounts reproduced in our plates in this book. Thus, the object of staining material to see if any bacilli of tuberculosis exist, is to reveal their appearance in a striking, unmistakable manner, in contrast with the decolorized germs of other character that may be, and usually are, present; or in contrast, sometimes, with a background, and with foreign germs stained differently. The true germs of consumption are set off more or less boldly, in a distinct tint of their own, according to the methods used. Most staining methods now used reveal the bacillus in a shade of red on a background of different color, green, blue, etc. The author's process always stains the bacilli red, and the field and sometimes some other germs a fair green, so that a mistake is not possible.

IV.— MOUNTING — GENERAL IDEA.

TO study any fine organism with a magnifying glass, it is not only necessary to increase its appearance in size, as one does letters by reading through an ordinary lens, but it is also necessary that light be reflected through it or through its immediate surroundings. This is accomplished by the direction of the rays of sunlight or lamplight through the object to be examined, by a little mirror attached to the microscope ; so the object must be very thin.

In searching for the bacillus of tuberculosis for diagnostic purposes, in 98 per cent of cases the material is fluid or nearly so, such as sputum, which furnishes at least 95 per cent of all specimens analyzed.[1] A particle of this is spread thinly on a clean, thin cover-glass,— the cover-glass known as number 1 or 2,— allowed to dry, then stained, washed, decolorized, and washed again, and then pressed, smeared surface downward, on a drop of water in the center of a clean glass slip, and examined at once with a microscope. Or, after washing it, the thin cover-glass may be dried by pressing it between two sheets

[1] I do not intend to go into details of analyzing the bacilli in tissue. This requires more skill and knowledge of histology and pathology than could be given here. It is rarely needed for diagnosis of tuberculosis

of clean, smooth blotting-paper or rice-paper (cigarette wrapper), then pressed on a small drop of Canada balsam instead of water, in the center of a glass slip. In the latter case, the mount should be held with the pincers, and heated slowly by passing the slip, cover-glass upward, to and fro over the blaze of a gas-jet or an alcohol lamp, or over a lamp chimney, until the balsam makes little bubbles by cooking between the slip and the cover. Then press the cover on the slip by gentle pressure with the pincers. Such a mount, when cold, will be glued tight,— cover-glass to slip,— and will be permanent. A water mount is temporary, but it may be made permanent (if found good on examination) by abstracting the water from it with absorbent paper in the manner just explained, and then mounting in balsam.

If the material be smeared too thickly, the stain will be so dense that light will not penetrate it well, and the mount will usually be found unsatisfactory. A few trials will indicate the amount of material required. In analyzing sputum, a little particle that will adhere to the point of a needle spread very thinly and as evenly as possible, will usually be found sufficient. A good-sized cambric needle, fixed at the end of a penholder or something of the kind, is very handy in taking up particles of sputum or any other such substances, and spreading them on the cover-glasses. Some prefer a very fine, smooth

scalpel or knife-blade. Others prefer a special sputum spreader. Others, again, press a particle of specimen between two cover-glasses, held between the thumb and finger, and thus spread it over the two surfaces brought in contact, pulling them apart, after gentle friction for a moment. It matters not how the spreading is done ; the thing is to spread a *thin* film on the cover-glass. In other words, in the general process of mounting to analyze fluid or semi-liquid substances for tuberculosis,—

First, clean the cover-glasses thoroughly. If they are cloudy, leave them in alcohol for a day or two, then very carefully wipe dry with a clean old cotton or silk cloth, or a thin piece of chamois skin, and inclose them in a clean box to use when needed. They may, however, be kept constantly in alcohol, and wiped clean before using.

Secondly, clean glass slips or slides likewise, and inclose them in a box, or wrap in paper to use when needed.

Thirdly, when a specimen is on hand, smear a very thin layer over *clean* cover-glass, and let it dry face upward, and pass once or twice rapidly over a blaze to coagulate the matter.

Fourthly, stain as per directions to be found elsewhere in this book.

Fifthly, decolorize in order to get out all the *excess* of stain possible from

the background, and even from the germs themselves, as is practicable by the use of special agents spoken of elsewhere; also wash in water.

Sixthly, if you wish (it is not indispensable), you may clarify the field by putting a drop of clove oil on the stained surface, after drying it well, and before mounting in balsam.

Seventhly, mount on the glass slip in a small drop of Canada balsam.

If a water mount is desired, put the cover-glass, after the fifth process, directly on a little drop of water on a glass slip, and examine.

V.— COLLECTING SPUTUM, ETC.

DR. SEVELIEFF recommends mixing the fresh sputum with 95 per cent of alcohol in order to preserve it. He suggests that expectoration be made directly into the alcohol. This, in fact, does preserve specimens, but it coagulates the albumen,— a condition which necessitates dissolution, with a two per cent solution of caustic potash, before spreading over a cover-glass for the purpose of staining and mounting. The author prefers to mix the sputum with a saturated solution of borax, which is recommended by Kuhne to liquefy the mucus before examination. A small, wide-mouthed bottle — say a quinine vial — is cleansed, filled about one third or one fourth with the solution, and well corked. It is then left with the patient with the request to expectorate directly into the vial, the first and second expectorations in the morning. The bottles distributed among one's patients ought to be labeled, numbered, and recorded in a book; and when the analysis is made, the results may be written opposite the right name. One could thus watch the weekly increase or decrease of bacilli under a particular mode of treatment.

The method of collecting sputum in paper boxes, envelopes, pieces of paper, and mailing the same to experts for examination, is a dangerous practice, which no conscientious physician should be guilty of. The writer has received such samples ; on being opened, the dried sputum broke or crumbled, and flew in a powder sometimes, thus endangering all in the immediate neighborhood.

The remainder of specimens which have been sufficiently analyzed, should be burned, or immersed in a solution of corrosive sublimate 1–1,000 for a few hours.

Other tuberculous matters may be collected the same as sputum, but chiefly in alcohol, and should be treated accordingly before examining.

VI.—STAINING FLUIDS, AND THEIR USE.

IN order to appreciate and differentiate the bacilli of tuberculosis, various staining solutions and methods of staining have been recommended. Unfortunately, most of them are better for experts than for busy practitioners.

No. 1. — Koch's Original Stain.

Concentrated alcoholic solution of methylene-blue, 1 part; 10 per cent potash sol., 2 parts; distilled water, 200 parts.

Float on, or immerse the preparation in, this stain, cold, 24 hours, or 1 hour at 104° F. (40° C.) Rinse in water; immerse in a watery solution of vesuvin for two minutes; rinse again in water, put on glass slip (slide), and examine. Or, treat with alcohol and clove oil, and mount in Canada balsam, as indicated elsewhere.

No. 2. — Gibbes's Double Stain.

Rosaniline hydrochlorate, 2 parts; methylene-blue, 1 part; triturate in a glass mortar. Dissolve 3 parts aniline oil in 15 parts rectified spirit, and add slowly to the above. Lastly, slowly add 15 parts distilled water. Keep in stoppered bottle.

Heat a little of the solution in a test-tube[1] until the steam rises, pour into a watch-glass, drop the cover-glass preparation in it, and allow it to remain for five minutes. Wash in methylated spirit till no color comes away. Dry in the air or over a spirit-lamp, and mount in Canada balsam.

No. 3. — Paquin's Three Solutions.

A. Fuchsin, 15 parts (or 15 grains); absolute alcohol, 120 parts (or 2 drachms); water, distilled, 480 parts (or 1 ounce). Dissolve the fuchsin in the alcohol and add the water.

B. Distilled water, 480 parts (or 1 ounce); liquor ammonia, 2 to 3 parts (or 2 to 3 minims). Mix and shake well.

C. Alcohol, 720 parts (or 1½ ounces); water, 360 parts (or 6 drachms); nitric acid, 30 parts (or ½ drachm), aniline, green, to saturation. Dissolve the green in the alcohol, stir, add water, and then acid.

These solutions, which have been evolved from several formulas and from personal experience, — principally the formula of Pittion and Roux, — though they consist of several ingredients, are more easy and simple to use than any

[1] To heat stain in a test-tube, pass the tube in and out, and revolve it in the blaze of a gas-jet or an alcohol lamp, or over a lamp chimney. It may be held with the fingers, near the mouth.

with which the writer is acquainted. Once made, which takes but a few minutes,[1] they fill every want in the staining process. No alcohol, no nitric acid, no ammonia, no other reagent of any kind is needed; nor is one obliged to make up stains and counter-stains every time sputum is to be examined as in certain processes. It stains admirably.

To use, it is only necessary to put about equal parts of solutions A and B (say a half teaspoonful of each) in a test-tube, heat it over a lamp till it boils or till vapor rises, empty in a watch-glass, drop the cover-glass preparation in it, and in five or six minutes — while the glass slip is being cleaned and prepared — it is sufficiently stained. Then rinse it in water, put a drop of solution C on it, rinse again; put on another drop of C if the mount is not a distinct green; wash again, and it is done. Put on a glass slip, and examine directly; or mount in balsam. These stains have revealed bacilli to me many times when all other methods had failed. They are very reliable, and usually give such a clear field that a $\frac{1}{6}$ objective, or a No. 7 of Leitz, is sufficient for diagnosis; whereas, by the other methods such a low power is not always reliable.

[1] They may perhaps be purchased already prepared and tested more cheaply than one can make them.

No. 4. — PAQUIN'S TWO SOLUTIONS (a modification of No. 3).

A and B of the above combined and modified thus: Fuchsin, 20 parts (or 20 grains); carbolic acid, 6 parts (or six minims); liquor ammonia, 2 parts (or 2 minims); glycerine, 15 parts (or 15 minims); alcohol, 120 parts (or 2 drachms); water, distilled, 960 parts (or 2 ounces). Dissolve the fuchsin in the alcohol; add the water and the other ingredients while stirring. To use, heat about a half teaspoonful in a test-tube until steam rises, put in a watch-glass, drop the preparation in it, and after five or ten minutes wash in water, decolorize, and color the field green in solution C of No. 3, as explained on the preceding page.

The following chapter gives the *modus operandi* of the writer in his daily examinations of sputum. It would take too much space to explain all the details, but it really takes less than ten minutes to do the work. It can be accomplished in five or six.

VII.—MODUS OPERANDI.

THE methods which the author has evolved out of several processes of staining the bacillus of tuberculosis for diagnostic purposes, give the greatest satisfaction. It is a fact that most all processes in vogue, though excellent when once well understood, and used by one who has had more or less experience, are very frequently disappointing to the beginner and the busy practitioner. Try as he may, he often finds no bacilli where an expert succeeds. Indeed, nearly all are better adapted for the trained microscopist or bacteriologist than for the busy man or student, because they usually give satisfactory results only on condition of tedious and exceedingly great care in details, and of knowledge of the minute but important changes in staining, decolorizing, etc. The following process avoids all these difficulties; it stains the bacilli red and the field green readily, without any expertness; in fact, one might say, despite carelessness (which, however, should be foreign to the character of a diagnostician).

1. If the sputum is dry, wet it. Then if it is gluey, or thick, or stringy, — conditions which preclude the possibility of spreading a very thin layer of it on a cover-glass, — mix a portion or all the specimen with about equal parts of a saturated solution of bi-borate of soda (borax), and shake well to make a uniform semi-liquid mass. This can be done in a test-tube (small preferred), or one may crush and mash a particle of sputum, and mix it well enough in a watch-glass, by the aid of a glass rod or something of the kind.

The use of this solution is always a wise precaution, to avoid errors of diagnosis, which are common with thick mounts. In case of nummular (cheesy) matter, it may be treated in like manner with a saturated solution of carbonate of ammonia. If the sputum is putrefied, it is liquid enough, and needs no such treatment, though, possibly, it may be unsatisfactory in other respects. If the specimen was preserved in alcohol, take a small lump of the clots formed, and liquefy it in a drop or two of a two per cent solution of caustic potash, either in a watch-glass or directly on a cover-glass.

2. Hold cover-glass between the thumb and index-finger of the left hand, spread a thin layer of specimen over its surface in the manner indicated in a preceding chapter, take hold of the cover-glass with fine forceps or pincers in

the right hand, and pass it rapidly over a blaze to and fro once or twice, to coagulate and dry the material on.

3. Put in a test-tube about equal parts, say a half teaspoonful, of solutions A and B of stain No. 3, or only of A of No. 4, of the foregoing list of stains, and heat by moving it over the blaze of a gas-jet or alcohol lamp, or the mouth of a lamp chimney, until it boils, or vapors rise. Then empty the solution into a watch-glass, and drop the cover-glass, smeared face downward, in it, and leave it there at least five minutes.

4. While it stains, clean a glass slip thoroughly, and holding it horizontally between the thumb and index-finger of the left hand, deposit a small drop of Canada balsam in the center, on one side, by pressing it out of the balsam tube gently with the right hand, and lay down the slip, gummed face downward, letting one end rest on something, so that the slip will make an inclined plane, and the balsam will *not touch the table*. By keeping this gummy surface under, one avoids the gathering of dust thereto.

5. Now take the cover-glass with pincers from the stain, and wash under a stream of water (from wash-bottle, faucet, or something else) until the water falls free from red tint. Then drop on it (the preparation), with a medicine

dropper, sufficient of solution C to cover the smeared surface, hold a few seconds, wash again, and if the tint of mount is still reddish or violet, even in spots or streaks, put on more of solution C, and again wash under a light stream of water. Continue this operation until the water comes off clear, and the smeared surface is green. The thinner the preparation, the sooner this will be accomplished; the thicker it is, the greener and darker the background, and the harder to get a clear field. As a rule, two successive treatments with solution C, and as many immediate washings, are sufficient. This need not take longer than a minute or two.

6. Hold the cover-glass, edge downward, on absorbent paper to drain, then put it on a clean sheet of such paper (or cigarette-paper, say), stained face upward, and dry it with a fold of the same leaf, or with another, in the manner of using a blotter on an ink stain. After this, if the preparation is too dark and thick, it may be clarified by the addition of a drop of clove oil on its stained face, this being expunged afterward, as if it were water, by the use of bibulous paper. The use of clove oil is not very safe, as it sometimes fades the stain of bacilli a little, and may cause errors. Use it quickly.

7. Now lay the cover-glass, stained surface upward, on a sheet of white paper (say a dry corner of the same bibulous paper used, if you please), take

the glass slip (slide) containing the drop of Canada balsam, and apply this gum directly on the stained face, so that the smeared surface of the cover-glass will come in contact with the drop of balsam, in juxtaposition in the center of the slip, and turn it over so as to bring cover-glass upward.[1]

8. Take the glass slip with pincers by one end, hold it with cover-glass upward, pass it to and fro, rapidly at first, and then slower and slower, over a blaze from gas or lamp, in order to melt down the thick balsam. Heat until it begins to boil, and little bubbles begin to raise the cover-glass from the slip. Then put the slide down (not on paint), cover-glass upward, and press the latter down with the pincers. It is then ready for examination. Canada balsam thus cooked becomes hard immediately after cooling.

9. If on examination it is found interesting or useful, it may be labeled and stored away as part of a collection.

It will be observed by some, that several of the details of this technique

[1] In case only a temporary mount is wanted, No. 7 is omitted, and the cover-glass is put, smeared face down, directly on a drop of water in the center of a glass slip, and examined at once. In case clove oil is used, one cannot thus mount in water. If one forgets which side the cover-glass and the material is on, scratch both sides of a colored spot with the point of a needle or pin or penknife blade. This will make a streak on the right side, and nothing on the smooth side.

are not strictly orthodox, that it is not in every respect just as many of us were taught. This is true; but it has the advantage of being simple and accessible to all. It removes many of the usual dangers of dirt, and soiling with dust and various kinds of flying particles; it stains rapidly, is reliable for diagnostic purposes, and finally it gives one a permanent mount within seven to ten minutes.

However, the *most reliable*, *striking*, and *beautiful* mounts are obtained by leaving the preparation in the red stain about ten minutes.

VIII.— STAINING BACILLI OF TUBERCULOSIS IN MILK, PUS, ARTICULAR SECRETIONS, ETC.

MANY fail in this because of bad methods. A writer unknown to the author, suggested, in the *Monitore dei Farmacisti*, the saponification of the fat globules as follows : —

"A drop of the milk is placed on a glass slip, and two or three times its volume of a one per cent solution of sodium carbonate is added, and the fluids mixed with the aid of a platinum wire. The slip is then cautiously held over the flame of an alcohol lamp, or over the chimney of a kerosene lamp, and the liquid slowly evaporated to dryness. During the evaporation, the butter particles are saponified, leaving a thin layer of desiccated soap on the slip."

After this, stain by the method recommended in the preceding chapter for the staining of tuberculosis bacilli in sputum.

None of the processes recommended to precipitate the casein in milk before staining and mounting, are satisfactory or reliable. As to bacilli in *pus*, *sticky or viscous* or *thick secretions* from articulations, etc., various

methods should be resorted to before forming an opinion, when the trials are negative. One should treat these matters as sputum or milk, with alcohol, or a borax solution, or a caustic potash solution, or with bicarbonate of soda, using these ingredients in the proportions and manner indicated elsewhere. The final object is to dissolve or liquefy the substance, and free the germs, so that they will take stain and reveal themselves.

Matter from an *ulcer*, such as scrapings from a suspected lupus, may be treated with a two per cent solution of caustic potash, and mashed thoroughly in it with a pestle in a small mortar, or with a glass rod in a watch-glass. The former is preferable. If one succeeds in getting a semi-liquid mass, a particle of it may be mounted as sputum. *Bowel discharges* need to be diluted, if too solid. The traces of mucus, or streaks of blood or pus, or spots containing viscous matter, should be chosen. If too thick, treat with some or other of the substances mentioned for the liquefaction of material, as explained for other substances, until the desired results are obtained, remembering that caustic potash liquefies coagulated albuminoids, and bi-borate of soda emulsifies, as it were, the fresh mucous matters.

Thin fluids, as *urine* and *pleuritic effusions*, need no special preliminary treatment.

IX. — HOW AND WHAT TO SEE THROUGH A MICROSCOPE.[1]

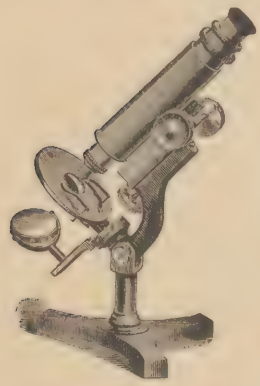
EVERYTHING has a beginning, and so many students, and even physicians, have yet to take their first peep through a magnifying tube such as is used in medical practice. To these a word on the subject may not be amiss.

In the first place, it is well to bear in mind that the higher the lens is capable of magnifying, the closer to the object it must be adjusted. So a lens known as a $\frac{1}{6}$ is a low power as compared with the lens known as a $\frac{1}{12}$, and the latter needs to be approached to within a much shorter distance of the object than the former. This is the focal distance; and the longer it is, the easier it is to focus properly. So one must act cautiously, according to the power used; for a careless or rapid screwing down or pushing of the tube to a focus, may strike the object and glass slip on which it is fixed, and possibly ruin it and the lens at the same time.

We will suppose that the microscope represented by the accompanying cut is now before you. A mounted slip, stained, is placed on the stage (platform)

[1] With special reference to tuberculosis.

beneath the lower point of the tube (objective), one eye looks through from above the eye-piece, the mirror underneath is revolved until the light[1] strikes the object fully, and the tube is *slowly* pushed down with the right hand, or screwed down, as it were, according to whether the microscope has a rack and pinion or not (this cut has them). At last the color of the field will appear. At this moment, stop lowering the tube with the coarse adjustment, and instead continue the focusing by revolving the fine adjustment screw — the small one[2] — now to the right, then to the left, if the vision indicates it, until a perfect outline of the object or objects sought is discernible.



If one is looking for the bacillus of tuberculosis with any objective, many other things than this will appear first. Perhaps dirt or spots of stain on the outside of the cover-glass will first be seen. The layer spread on the glass,

[1] Bright daylight is the best. A northern window is preferable. Lamplight may be used to advantage, and will reveal fine bacilli better than indifferent daylight.

[2] It is underneath in this cut.

no matter how thin, when measured microscopically, has a certain relative thickness, and the objects nearest to the lens will be revealed first; if the tube be lowered a trace more, the things directly underneath the first ones seen will appear. So in moving the tube the hundredth or fiftieth part of an inch say, up and down, after the glass is focused, one may perceive different things lying superposed in the thickness of the layer of substance examined. Besides this the microscope will reveal coarse and comparatively large objects, such as specks of dirt for instance, which may appear like dirty rocks; or a cotton thread, which may look like a broad, ragged tape, much sooner than the incomparably smaller objects, such as the bacillus of tuberculosis. With a low power, one must look sharply to see these.

Sometimes the outside surface of cover-glass is not clean, or is stained a little; when the lens comes to it, the beginner stops; he is disappointed to find only blurred, dirty spot crystals, cotton threads, or unstained or stained patches of dirt, and perhaps even germs (of the air or substance analyzed). These are outside the cover. Always be sure that the mount to be examined is first wiped with a clean cloth. If the dirt will not rub off, dip a kerchief in alcohol, and gently wash it from the cover-glass, at least.

Having focused the lens properly, look sharply, coolly, intently, over

the field, then deliberately and slowly in each doubtful spot. If no bacilli are visible after a good survey of the field, move the slide *very*, VERY, VERY slightly with the left hand while focusing up and down with the *fine screw* or tube adjustment with the right.[1] At first, the unsteadiness of the hand moving the slide, may jerk the field clear out of sight, and cause a feeling of nervousness, impatience, and even anger to arise in the heart. I have seen students utter imprecations at the unsteadiness of their own hand, when a beautiful field was suddenly removed from their gaze, not to be found again until after hours of search. But this only spoils the nerves, and makes matters worse. An hour of practice in moving a focused object under a microscope, while looking at it, will make one master of it sufficiently for practical purposes, and every examination adds to the skill in manipulations. A smeared cover-glass of sputum may be only a half inch in diameter; but when it is under the microscope, it is a vast field, and one should not weary or be at once discouraged if the object of search is not found. One must look carefully all over the field by moving the slide as methodically as possible, first one way, then another, with the fingers (or a mechanical stage such as some microscopes are provided with) until the whole is surveyed, if necessary. If one, two, or even three

[1] Move the slide with the right if it is easier, and focus with the left.

mounts of a given specimen fail to reveal the germ, it may be wise to make still another and another, and examine carefully. As a rule, in cases where one fails to find the bacillus the first time, another specimen is examined a day or two later; and if this reveals nothing, examine another, and then another, at various intervals (of days or weeks), until the parasite is found, or the indications point to its absence.

The pus cells, mucous debris, dirt, cotton fibers from the cloth or kerchief used, blurred patches, etc., need not be considered, except as a curiosity, to indicate improvement in mounting. Other germs should be left entirely unnoticed, at least until the particular one sought is discovered or believed to be absent. The knowledge of the presence of these other microbes, has no positive value, from a diagnostic point of view, in tuberculosis.

X.—THE BACILLI OF TUBERCULOSIS.

THESE little rod-shaped parasites vary between 2 and 6 μ[1] in length, according to special conditions of their life and 0 μ 3 to 0 μ 5 in width. Their average length is about 3 μ, and the average width about 0 μ 4. They may be straight, or slightly curved, or irregular in their aspect. They appear either as homogeneous little rods, or as finely dotted bacilli; these dots are due to the presence of fine ovoid or spherical bodies placed end to end like a very minute string of beads. These germs can scarcely be seen without coloring reagents, except with high-power lens, and then the dots, or spherical bodies, which are looked upon as spores, do not appear. The organisms unstained, particularly if treated with potassa, have the appearance of hyaline, or motionless rods.

The stained bacilli of tuberculosis can be appreciated when magnified 350 to 400 diameters, but it is better to examine them under greater increase, say

[1] The μ (mikron), a unit in certain microscopic measurements, means micromillimeter, and is equal to one-thousandth of a millimeter. A millimeter is, as the word implies, the one-thousandth of a meter, and a meter is equal to 39.37 inches. A millimeter, then, is nearly 1–25 part of an inch and μ (mikron) is the 1–1,000 part of 1–25 of an inch.

480, and it is still better to see them at 700 or even 800 if possible. This high increase is not necessary for diagnosis, if good stains are used.

The bacilli may appear shorter or longer in the same specimen, according to their age. For instance, fresh sputum may at first show very fine short rods. Let it stand a day or two, and if it does not desiccate, the forms will be found more clearly dotted and longer. After standing for days or weeks, many fine, loose dots — spores doubtless — may be observed, and long bacilli formed by them, disposed end to end. In patients having large lung cavities, the bacilli are sometimes longer, and the loose spores more numerous, than in milder cases.

The quantity of bacilli in sputum, and in all fluids, varies greatly; it depends on the extent and age of the lesions. They are usually much more numerous in extensive cavities than in secretions from small lesions resulting from slight tubercles. A given mount may contain only a few — half a dozen or less, and the next, hundreds.

Sometimes we find bacilli in a large cell, a giant cell, indicating an attempt, perhaps, on the part of nature, to destroy the germs by cellular digestion — phagocytosis.

Usually specimens to be analyzed, particularly sputum, contain numerous

other germs which have no direct relation to the disease so far as the cause is concerned, but are doubtless very harmful complications; for they must be, as in wounds, dangerous and most potent factors in the destruction of tissue, in producing pus cavities, and in creating very damaging ptomaines.

The good influence of high altitudes on consumptives, is due not a little to the fact, I imagine, that pus or decomposition germs are comparatively rare in high altitudes, and complications such as almost always exist in other atmospheric conditions in tubercular lesions opened to the air, find there no destructive agent of the class of ptomaine makers and the like. These foreign germs, in making analysis, can be made to appear unstained at the side of stained tubercle bacilli, or may be stained as the background, and may therefore be readily distinguished.

Tubercle bacilli may be found in sputum, milk, urine, tubercular abscess of the skin, joints, etc., and also in intestinal discharges. In cases of these discharges, one may discover the bacilli in analyzing the liquid of an evacuation obtained by a clyster, given a few minutes after a thorough washing of the intestines by careful but extensive enema. At other times it is necessary to analyze much of the fæcal matters, and frequently to repeat the operation. Hundreds of other germs may be found in these matters.

XI.—REMEMBER:

THAT the presence of the bacillus of tuberculosis in any organ *always* indicates the presence of a field in which it grew—a lesion of some sort, considered justly a symptom of consumption, whatever name man may give to this disease; but—

That the *absence* of the bacillus from sputum, milk, urine, and fæces, is *not always* an indication of the absence of the disease, as it is necessary that a lesion be broken open, and that a secretion come from it, before it is possible that bacilli be expelled.

That in the earliest stages of lung tuberculosis, cough and mucus from the bronchials, and even from points irritated by the presence and action of bacilli, may precede the expulsion of the latter by many days, weeks, and even months.

That when coughing or other symptoms of pulmonary consumption are present, analysis of the sputum should be made daily, or at least two or three times a week, until a satisfactory diagnosis can be arrived at.

That tuberculosis should be considered transmissible, and the specimens after testing should be burned, and never allowed to desiccate in a vessel or anything else. Nor should instruments used, be used again without sterilization, *i. e.*, cleaning by steam, or washing in boiling water, or heating over the blaze of an alcohol lamp or some other fire.

That it is a grave responsibility that a physician has, in handling cases of consumption or suspects thereof, and microscopy is often the most reliable means of diagnosis.

That every other means of diagnosis, such as family history, patient's history, etc., should be considered in evidence, and the microscope used as an expert witness.

That in doubtful cases, a *few* rabbits or guinea-pigs may be inoculated hypodermically, and in the peritoneum, with fresh sputum, and thus all doubts be removed by the production of the disease, or the reverse result. By inoculation of virus in this way, quantities of sputum thousands of times larger than can be seen under the microscope by a hundred analyses, may be used, and several bacilli or spores that the microscope might not have come across, be inoculated.

That an expert may readily see the bacilli of tuberculosis in sputum with a $\frac{1}{6}$ objective, and that a beginner may fail to see them, even with a $\frac{1}{12}$. Therefore the eye should be trained by exercise.

That a sub-stage condensor attached to a microscope is an admirable aid in concentrating the light to study fine objects, such as bacteria.

That abscesses produced by inoculation of sputum, arising within a week or so, followed by more or less pus and perhaps suppuration, is not of itself an indication of tuberculosis. The characteristic tubercles must be found, and also the germs, to conclude affirmatively.

That one may often mash and macerate a meaty tubercle (thereby avoiding sections), and by mounting the pulp as sputum, find the bacilli, even in cases artificially produced in animals.

XII. — DO N'TS.

DO N'T hasten in mounting. You will waste stain, break glass, stain badly, and perhaps injure some instrument.

Do n't jump at conclusions in the negative or affirmative, when the revelations of the microscope are not *clear cut* and *positive.*

Do n't guess at a diagnosis, in a matter of such grave responsibility and great magnitude. It is wrong. A mistake may send a father, mother, or beloved child to the grave.

Do n't neglect or discard other means of diagnosis because you use a microscope. This instrument is an expert witness, whose affirmative evidence always indicates consumption, but whose failure to reveal the germ does not in all cases — though it does in the majority, after repeated tests — indicate the opposite.

Do n't forget that the determining cause of tuberculosis is doubtless the bacillus, but that bronchitis, *La Grippe*, so-called bad colds, etc., may prepare the field, or may be, in other words, the exciting cause.

Do n't accept, for the bacillus of tuberculosis, any germ having *its* appear-

ance, unless it clearly presents itself in the proper shade and form, as indicated by the particular staining method used. For instance, if the author's process is used (see Nos. 3 and 4, staining methods), the background should be a good green, the bacilli red, and the other germs unstained or pale, or stained a faint green, or indistinctly blurred with shades bordering on these tints, such as purple.

Don't forget that these staining processes are all differentiating in their effect, and should stain distinctly only the bacillus of tuberculosis.

Don't forget, either, that a beginner may fail to get, at first, uniformly distinct shades between germs and background, because of insufficient decolorizing or washing, or a too thick layer of material on the cover-glass. In such cases, the thinnest parts will have a fair appearance, but the thicker spots will appear somewhat blurred, violet or purple or dark reddish, and in them the differentiation of germs is not satisfactory. Such spots are *unreliable* points of study.

Don't be puzzled when bacilli of tuberculosis in one case are very large and plain, and in the next so fine and thin as to be barely discernible with certainty; such is often the case. It is only a difference in size, etc., due to a better or poorer soil. In this respect, it has valuable indications.

Don't dismiss the thought of tuberculosis when you find only red spots or specks on a different colored background,— say green,— for they may be groups of bacilli or spores. Search farther; make more mounts.

Don't pay attention to bacilli stained the same color as the background; they are not bacilli of tuberculosis.

Don't forget that spores in fresh sputum are exceedingly rare; in old sputum they may be very numerous.

Don't forget that one may search daily for months in the sputum of one individual, and find no bacilli of tuberculosis, and then suddenly find a perfect mass of them. This is due to a sudden breaking of a tubercle or several of them.[1]

[1] The writer once examined very closely, two or three times a week, during two months, the sputum of a patient, for bacilli, and failed to find any. Two weeks after he stopped, another person made a single examination, and found several. The author then made another examination, and also found several.